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94.04.22 94DE-4414185(+94DE-4401451) *(95.07.27)* C12N 15/61, 1/21, 9/24, 9/26, C12P 19/24, C12N 9/90, 15/56 (C12N 1/21, C12R 1:01, 1:18, 1:19)

Sequences for proteins with sucrose-isomerase activity - and cells producing increased amts. of palatinase and trehalulase; useful for the prodn. of non-cariogenic sugars (Ger)

C95-135612 N(AU BR CA FI JP NO US) R(AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE)

Addnl. Data: MATTES R, KLEIN K, SCHIWECK H, MUNIR M, KUNZ M
95.01.18 95WO-EP00165

Issued in Week 9540.

First Major Country Equivalent to NO9500194.

DNA sequences as follows, also sequences homologous or complementary to them, are new:

(A) a sequence encoding a protein with sucrose isomerase activity and comprising one of the six nucleotide sequences given in the specification (i.e. 1890, 1305, 471, 1803, 1794 and 1782 bp), opt. without their signal peptide coding regions; and

B(4-E2E, 4-E2F, 4-E8, 4-F10, 4-N4) D(5-C3C, 5-C8, 5-H12A, 5-H12E, 5-H14A1, 5-H17A3, 6-G) .5

(B) a sequence encoding a protein with palatinase and/or trehalulase activity, comprising the 1362 or 1704 bp sequence given in the specification.

Also claimed are:
(1) vectors including (A) or (B),
(2) cells contg. (A), (B) or a vector as in (1), pref. being *E. coli*, *Protaminobacter rubrum* or *Erwinia rhapontici*,
(3) proteins encoded by (A) or (B) and having (a) a sucrose isomerase or (b) palatinase and/or trehalulase activity, respectively; and
(4) cells contg. DNA for a protein with sucrose-isomerase activity and having a reduced palatinose and/or trehalulose metabolism.

USE

The proteins with sucrose-isomerase activity or cells contg. (A) are used in the prodn. of a non-cariogenic sugar (claimed), in particular of trehalulose and/or palatinose.

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ADVANTAGE

In contrast to prior art methods for the isomerisation of sucrose to trehalulose and/or palatinose, the formation of monosaccharides is largely avoided. The new organisms achieve a larger yield of palatinose and/or trehalulose.

SPECIFICALLY CLAIMED

The plasmid pHWS88 (DSM 8824) and the mutant *P.rubrum* transformant SZZ 13 (DSM9121) are specifically claimed.

PREPARATION

DNA coding for a protein with sucrose isomerase activity was isolated from a donor organism library using standard gene cloning techniques. In particular, the library is screened with a probe sequence amplified from the donor organism using the following primers:

5'-TGGTGGAARGARGCTGT-3';
5'-TCCCAGTTCAGRTCCGGCTG-3'

EXAMPLE

Sucrose-isomerase genes were isolated from *P. rubrum* DNA (Sau3A cleaved, screened with 5'-ATCCCGAAG TGGTGGAAG GAGGC-3', isolation of pHWS88) and *E. rhapontici* (screened with 5'-TGGTGGAAA GAAGCTGT-3' and 5'-TCCCAGTTC AGGTCCGGC TG-3'). A palatinase defective mutant was produced from *P. rubrum* (according to Miller, J. Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, 125-179 (1972)).

Mutants were white on MacConkey-palatinose medium (Difco Laboratories, Detroit, Michigan, USA) and accumulate palatinase when growing in minimal medium with 0.2% sucrose (mutant SSZ, DSM9121). SZZ did not contain any glucose or fructose compared to the wild-type cells contg. 2.6% fructose and glucose in the total cell and 123% in the raw extract. (GS1).

SR: No Search Report.

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